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EVALUATION OF YEASTS AS PLANT GROWTH PROMOTER FOR RICE (ORYZA *SATIVA L.*), PEPPER (*CAPSICUM ANNUUM L.*) AND TOMATO (LYCOPERSICON *ESCULENTUM* L.) SEEDLINGS

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June, 2017

Gondar, Ethiopia

EVALUATION OF YEASTS AS PLANT GROWTH PROMOTER FOR RICE (*ORYZA SATIVA L.*), PEPPER (*CAPSICUM ANNUUM L.*) AND TOMATO (*LYCOPERSICON ESCULENTUM* L.) SEEDLINGS

A Thesis Submitted to Department of Biology of University of Gondar in the Partial Fulfillment of the Requirements for the Degree of Master of Science in Applied Microbiology

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Approval Sheet

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LIST OF ACRONYMS AND ABBREVIATIONS

ACC = Aminocyclopropane-1-Carboxylate

ANOVA = Analysis of variance

CDR = Complete Randomized Design

CtHY = Candida tropicalis HY

IAA = Indole Acetic Acid

IM 42 = Indiyam 42

IPYA = Indole-3-pyruvic acid

IWUE = Intrinsic Water Use Efficiency

PDA = Potato Dextrose Agar

PGPR = Plant Growth promoting Rhizobacteria

PGPY = Plant Growth Promoting Yeasts

PSY = Phosphate Solubilizing Yeast

RP = Rock Phosphate

SD = Standard Deviation

SPSS = Statistical package for social sciences

YPD = Yeast Peptone Dextrose

ABSTRACT

Yeast populations are usually higher in the rhizosphere as opposed to the bulk soil and this has increased the association between yeasts and different agricultural crops. The use of yeast as a growth promoter in agriculture has received considerable attention because of their bioactivity and safety for human and the environment. The aim of this study was to evaluate yeast isolates from different sources for their plant growth promoting ability for Rice (Oryza sativa L.), Pepper (Capsicum annum L), and Tomato (lycopersicon esculentum L.) seedlings. The study was conducted from December, 2016 to April, 2017 in University of Gondar, Gondar, Ethiopia.This study was performed by utilizing laboratory based experimental design. The study employed both qualitative and quantitative data for data analysis and the data recorded was analyzed using SPSS version 17 software and analysis of variance (ANOVA).The analyzed data were interpreted by using descriptive statistics, charts, tables, and graphs. Stem length (cm), number of nodes, number of internodes, and number of leaves, root length and fresh and dry weights of tomato, pepper and rice seedling varieties positively and significantly responded to yeast application. Rhizospheric soil yeast isolates (I5) were achieved the highest values ***(****17.45 cm stem length of Tomato Melksalsa****,*** *15.2g fresh weight of Pepper IM 42, 13.35 numbers of leaves of Tomato Melksalsa , 9.05 internodes and 9.35 number of node of Tomato Melksalsa* and *3.5g dry weight measurement of the Pepper IM 42****).*** *The root elongation efficacy by Isolate 5 (I5) were the highest percentage in Pepper IM 42 (57.25%), Pepper Markofana (55.08%), Rice x-jigna (50.40%), Rice ediget (48.275%), Rice Getachew (59.84%), Tomato melksalsa (55.17%), Tomato kochero (57.44%) and Tomato miya (69.46%). It can be concluded that yeasts effectively improved the growth of Tomato, Pepper, and Rice seedling varieties without causing any impact on the environment and on the ecology of other organisms. So this study will provide the community and farmers the cost effective type of organic fertilizer for the production of sustainable agricultural Rice (Oryza sativa), Pepper (Capsicum annum L.), and Tomato (Lycopersicon esculentum* L*) varieties by yeast.*

KEY WORDS / PHRASES: Pepper, Plant growth promoters, Rice, Tomato, Yeast

1. INTRODUCTION

Yeasts are unicellular fungi that can reproduce mainly through asexual reproduction and grow rapidly on simple carbohydrates, often through fermentative as well as respiratory pathways. As a result of their nutritional preference, yeast populations are generally an order of magnitude higher in the rhizosphere as opposed to the bulk soil. Diverse range of yeasts exhibit plant growth promoting characteristics, including pathogen inhibition, phytohormone production, phosphate solubilisation, N and S oxidation, siderophore production and stimulation of mycorrhizal-root colonization(Amprayn *et al.,* 2012).

Engaging in vegetable production, the chemicals of regulatory effect on plant growth and development (biostimulators) are one of means for obtaining the increase in plant performance. However, plant biostimulation has recently become an increasingly more common treatment in modern agricultural production, among such substances are plant growth promoting rhizobacteria (PGPR), yeasts, yeast extract and humic acid (Dawa *et al*., 2012). The use of yeast as a growth promoter in agriculture has received significant attention because of its content of many nutrient elements and productive compounds of semi growth regulator compound like auxins, gibberellins and cytokinins (Ahmed *et al*., 2011).

Many authors examined a wide diversity of soil yeasts for their potential as bio-fertilizers (Agamy *et al*., 2013; Nahed *et al.*, 2015; Amprayn *et al.*, 2012). Moreovere, because of the bioactivity and safety for humans and the environment. Yeasts become important kind of biofertilizers in soil fertilization or in foliar application on the shoots of vegetable crops. Many authors showed the effect of application of yeast on vegetative and fruit growth due to its richness in tryptophan which consider precursor of IAA (indole acetic acid) and on flower ignition due to its effect on carbohydrate accumulation (Khatab *et al.,* 2015; Fawzi *et al*., 2014).

Yeast as a natural growth promoter is also characterized by its richness in protein 47%, carbohydrates 33%, nucleic acid 8%, lipids 4%, and different minerals 8% such as Na, Fe, Mg, K, P, S, Zn, Mn, Cu, Si, Cr, Ni, Va and Li in addition to thiamin, riboflavin, pyridoxine, hormones and other growth regulating substances (El-Sayed and Eman, 2011).

Karajeh (2014) reported that the growth, disease resistance and the total yield of Tomato (*Lycopersicum esculentum)* were improved because of the application of yeast fungus *Saccharomyces cerevisiae*. Similarly *Saccharomyces cerevisiae* plays a beneficial role in cell division and cell enlargement of different plants and agricultural crops. Yeasts can also produce the auxin indole-3-acetic acid (IAA) and gibberellins which are known for their role in plant cell elongation, division, and differentiation (Nassar *et al.*, 2005).

As Ghoname *et al.* (2010) showed, spraying sweet pepper plants (*Capsicum annuum* L.) cv. with yeast solutions promoted plant vegetative growth i.e. plant height, number of leaves and branches, fresh and dry weights, individual fruit weight and number of fruits. Likewise, the application of soil yeast *Candida tropicalis* HY (CtHY) on germinated rice seedlings resulted in better rice plant root growth and the colonization of CtHY was confirmed to persist on plant roots at least for 3 weeks (Amprayn *et al*., 2012). The strain also tested positive for polyamine and phytase production, and mobilized phosphate from insoluble tri-calcium phosphate.

Promotion of plant growth was focused primarily using bacteria commonly known as plant growth promoting rhizobacteria (PGPR). As a result data on plant growth promoting yeasts are scarce or lacking. So, application of yeasts as plant growth promoter acts as a new trend for different agricultural crops. It is assumed that a better understanding for the role of yeasts in the promotion of growth of tomato, rice, and pepper will hold important role for future sustainable agricultural practices. Organic farming strategy is growing rapidly all over the world to conserve human health and the environment, which became under risk because of the unbalance use of pesticides and chemical fertilizers. Therefore this study is initiated to evaluate the growth promoting ability of yeast for Rice, Pepper, and Tomato seedlings to escape the danger that would happen at early stages.

2. LITERATURE REVIEW

2.1. Role of yeasts for plant growth

Yeasts synthesize antimicrobials and other useful substances required for plant growth from amino acids and sugars secreted by bacteria, organic matter and plant roots. Yeast as a natural source of cytokinins also stimulates plant cell division and enlargement as well as the synthesis and enlargement and synthesis of protein, nucleic acid (Agamy *et al*., 2013). Yeast extract is a natural component contains many of the nutrient elements and cytokininis, which is safe and non-pollutant. It has considerable amounts of amino acids, mineral elements, and carbohydrates, reducing sugars, enzymes and vitamins B1, 2,3,12. Also it is a source of cytokinins and protein that enhance cell division and differentiation (El-Bassiony *et al.*, 2014).

Many investigators reported that spraying yeast extract significantly enhanced vegetative growth performance and chemical composition of leaves (El-Tohamy *et al.*, 2008) on eggplant; (Ghoname *et al.*, 2010) on sweet pepper. Applying yeast to field bean plants increased contents of chlorophyll a, b, and total chlorophyll. Yeast extract gave the best values of total sugar content and its beneficial effect on carbohydrate accumulation in leaves of field bean (EL-Yazied and Mady, 2012).

Moreover, yeast extracts contain trehalose-6-phosphate synthase which is a key enzyme for trehalose bio synthesis. It also considered that the production of trehalose not only affects plant development but also improves drought tolerance (Asmaa *et al*., 2013). According to Nahed *et al.* (2015), using yeast as bio fertilizer can increase the ascorbic acid on sweet pepper fruits.The typical *Saccharomyces* spp, *S. cerevisiae also have* strong potential as plant growth promoters and as biocontrol agents of the soil-borne fungal plant pathogen *F. oxysporum* causing damping-off symptoms in sugar beet seedlings (Tolba *et al*., 2016). As mentioned in Hafez (2013) and Shalaby and El-Nady (2008), yeast in the soil, leaf area was more than two fold increased indicating enhanced cell division rate and cell enlargement and their length, diameter and fresh weight were also significantly enhanced.

2.1.1. Role of yeast for the growth of rice plant

Rice growth performance is subjected to environmental factors which affect the physiological processes inside rice plant cells. Improving rice physiological characteristics is considered to be

desirable due to its agronomic importance towards the achievement of high rice yield (Doni *et al*., 2014). The application of *Candidia tropicalis* HY (CtHY) on germinated seedlings resulted in better rice plant root growth and the colonization of CtHY was confirmed to persist on plant roots. Moreover, CtHY exhibits a number of common plant growth promoting characteristics and is capable of enhancing rice seedling growth when inoculated alone, rather than as part of a multi-strain inoculants (Amprayn *et al.,* 2012). In addition, Cong *et al.* (2009) found that the inoculation of rice with soil yeasts, significantly increased grain and straw yields and total N uptake as well as grain quality. According to Gao *et al.* (2014), the application of yeast alleviated the drought stress-induced adverse effects on the growth of rice plants by producing biologically active materials. Under drought conditions, yeast had a more promotive effect on production and they can improve rice drought tolerance and increase IWUE (Intrinsic water use efficiency).

2.1.2. Role of yeast for the growth of tomato seedlings

According to Lonhienne *et al.* (2014), supplying plants with brewer's yeast enhanced the N and P status of sugarcane and tomato plants and altered their growth and biomass partitioning. Similarly, N and P content increased in shoots of plants supplied with dead yeast (tomato) and living yeast (tomato and sugarcane). Furthermore, field application of *S. cerevisiae* improved tomato growth and yield and increased nematode resistance of tomato cv. Asala through increasing its root total phenolic content in a similar way as exogenously applied hydrogen peroxide (Karajeh, 2014).

2.1.3. Role of yeasts for the growth of pepper seedlings

Sweet pepper plant gave significant increase in yield and its components (fruit length and diameter, fruit fresh weight and dry matter percent in fruit as well as early and total yield) with highest quality for fruit (N, P, K% and Vitamin C) as the result of application of yeast (Nahed *et al.,* 2015). This study illustrated that the inoculation of sweet pepper with *Saccharomyces cerevisiae* and amended soil with compost tea, azolla extract led to enhancement of the plant growth, as yeasts are capable of directly enhancing the plant growth by the production of plant growth regulators. Also, the beneficial effect of yeast extract might be due to that it is considered as a natural source of cytokinins which stimulate cell division and enlargement as well as the synthesis of protein, nucleic acid and chlorophyll of a pepper plants (Dawa *et al.,* 2012).

As El-Fawy *et al*. (2015) mentioned, yeast treatments were significantly enhanced stem length and diameter of pepper plants compared to control plants. All tested yeasts were effective for increasing survival plants as well as improved plant growth characters compared to control pepper plants and the treated plants were very strong when compared to the control and fungicide. Moreover, all applied materials have positive and growth promoting effects on sweet pepper plants by providing supplemental doses of nutrients to the plants and in some cases to provide plants with some promoting growth regulators as well as yeasts (Ghoname *et al*., 2010).

2.2. Mechanisms of Plant Growth Promotion

2.2.1. Phytohormone production

The stimulation effect of active dry yeast on Grapevines growth might be attributed to its own higher content of amino acid and cytokinin and minerals as well as its positive action on enhancing the biosynthesis of carbohydrates (Fawzi *et al.,* 2014). For example, the typical Saccharomyces yeast *Saccharomyces cerevisiae* produce the auxin indole-3-acetic acid (IAA), gibberellins and plant hormones which has direct effect on the growth of plants (Nahed *et al.,* 2015).

The auxin indole-3-acetic acid (IAA) stimulates rapid and long-term responses in plants and has been identified in plant-associated yeasts (Sun *et al.,* 2014). IAA is a signaling molecule in certain microorganisms and modifies gene expression. According to Nassar *et al. (*2005), a plant-growth-promoting isolate of the yeast *Williopsis saturnus* endophytic in maize roots was found to be capable of producing indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA) in vitro in a chemically defined medium.

2.2.2. Siderophore production

Siderophores are vital for promoting plant growth and suppressing the effect of plant pathogens because of their iron-transporting abilities (Kloepper *et al.,* 1980). They are critical in ensuring low iron availability because iron is a critical plant growth limiting factor (Shih-Feng *et al.,* 2016). In addition, production of siderophores that chelate iron, making it unavailable for the pathogen growth, is critical for promoting plant heath.The non-reductive uptake of several siderophores (ferrioxamine B, ferrichrome, triacetylfusarinine C and ferricrocin) by various

strains of *Saccharomyces cerevisiae* was studied (Lesuisse *et al.,* 1998). Moreover; several aspects of siderophore transport were examined, including specificity of transport, regulation of transport and intracellular localization of the ferri-siderophores.

Siderophores have also been reported to be produced by species of *Candida* (Ismail *et al*., 1985) and *Rhodotorula* (Calvente *et al*., 2001). It is known that microbial siderophores provide plants with Fe nutrition to enhance their growth when the bioavailability of Fe is low whereas the exact mechanism is fairly unknown. Two possible mechanisms were suggested by which plants could obtain Fe from microbial siderophores: (i) Microbial siderophores with high redox potential can be reduced to donate Fe(II) to the transport system of the plant. In this mechanism, it has been hypothesized that the microbial Fe (III)–siderophores are transported to the apoplast of the plant root where siderophore reduction may be occur (Ahmed and Holmsrom, 2014).

2.2.3. Pathogen inhibition

Microorganisms in the substrate can be a great help in suppressing plant diseases and a great deal of research is being conducted in this area of horticulture. One study has shown that introducing beneficial micro-organisms and or adding compost to increase the amount of micro-life can have a major effect on crop performance (Karajeh *et al.,* 2014).

Agamy *et al*. (2013) emphasized that the mechanism in the biocontrol activity of *S. cerevisiae* include nutrient and site competition and induced resistance and/or make physical and chemical soil properties unfavorable for plant pathogens. The application of the yeast *S. cerevisiae* could suppress the infection and population of *Meloidogyne javanica* on tomato and increase its resistance and improve tomato root growth and yield under field conditions. Shalaby and El-Nady, (2008) also suggested that the potential of *S. cerevisiae* as biocontrol agent against *Fusarium oxysporum* F4 causing sever damping-off symptoms of sugar beet.

2.2.4. Yeast root colonization

Yeasts associated with the roots of plants have an important function in plant growth and in soil carbon sequestration. Yeasts in the root zone may influence plant growth indirectly by encouraging the growth of other plant growth promoting rhizo microorganisms, through vitamin B12 production (Agamy *et al.,* 2013). The increase of total microbial count, yeast and total

nitrogen fixer count populations in soil amended with organic fertilizer could be attributed to the act of simple organic compounds found in compost tea and azolla extract associated with root exudates of sweet pepper plants that are readily assimilated by yeast and other microorganisms (Nahed *et al.,* 2015).

Rhizosphere-competent yeasts were used to control *R. solani* diseases of sugar beet. In the root colonization plate assay, *C. valida* and *T. asahii* colonized 95% of roots by 6 days after radicle emergence, whereas *R. glutinis* colonized 90% of roots after 8 days. Root colonization abilities of the three yeast species tested by the sand-tube method showed that roots and soil particles attached to roots of 21-day-old sugar beet seedlings were colonized to different degrees by the three yeast species (El-Tarabily and Sivasithamparam, 2006).

The addition of live or dead yeast to fertilized soil substantially increased the nitrogen (N) and phosphorus (P) content of roots and shoots of tomato (*lycopersicon esculentum)* and young sugarcane plants. Yeast addition to soil also increased the root-to-shoot ratio in both species and induced species-specific morphological changes that included increased tillering in sugarcane and greater shoot biomass in tomato plants (Lonhienne *et al.,* 2014). These findings support the notion that brewers' yeast is a cost-effective biofertilizer that improves not only plant nutrition but also plant vigor during the early growth phase. It remains to be established which yeast-derived substances trigger the observed plant growth effects, and how rhizophagy contributes to plant nutrient acquisition.

2.2.5. Phosphate solubilization

Yeast facilitates the growth of plants by improving the uptake of nutrients and production of some phytohormones and convert insoluble form of phosphorous into soluble one enhancing phosphorous availability to plants. In addition soil yeasts might be used as inoculants to stimulate beneficial processes such as sulfur oxidation and phosphorus solubilization in soils (El-Bassiony *et al.,* 2014).

As Xiao *et al.* (2013) investigated, four yeast strains, *Rhodotorula* sp., *Candida rugosa*, *Saccharomyces cerevisiae* and *Saccharomyces rouxii*, which were isolated from wheat rhizospheric soils, showed high performance of solubilizing rock phosphate (RP). The yeast isolates demonstrated diverse levels of soluble phosphate releasing abilities in modified

Pikovskaya liquid medium containing RP as sole phosphate source. *C. rugosa* was the most effective solubilizer under different conditions, followed by *Rhodotorula* sp., *S. rouxii* and *S. cerevisiae*. The mechanisms employed by *Saccharomyces* spp. in enhancing nutrient availability by solubilization and chelation of minerals can increase plant metabolism leading to the enhancement of plant physiological activity (Tolba *et al*., 2016).

Yeast capable of solubilizing sparingly soluble phosphorus in pure culture have been isolated and studied. Several mechanisms such as lowering pH by acid production, chelation and exchange reaction in the growth environment have been reported to play a role in P solubilization by phosphate solubilizers. Such microbes not only accumulate P but a large portion of soluble phosphate is released in quantities in excess of their own requirement (Narsian *et al.*, 2008). Hesham and Mohamed (2011) reported that various efficient indigenous phosphate solubilizing yeasts were isolated from soils taken from different Egyptian geographic regions. One of the isolates, PSY-4, identified as *S. cerevisiae*, efficiently solubilized and released P from an insoluble form and successfully improved the shoot and root growth of corn plants.

Phosphate solubilization of soil yeast isolates were also evaluated in Pikovskaya's broth supplemented with different NaCl concentrations. This study revealed that all four yeast isolates solubilized insoluble Ca3 (PO4)2 under NaCl stressed conditions. Therefore, it seemed that they had been well adapted to the salt stressed conditions and they have genetic potential to solubilize the insoluble phosphate at high salt concentration (New *et al*., 2013). El-Mehalawy *et al.* (2004) also reported that rhizosphere actinomycetes and yeast fungi promoted plant growth by oxidizing ammonium to nitrate, oxidizing elemental sulphur to sulphate and solubilizing insoluble phosphate. The soil yeasts *Candida tropicalis, Geotrichum candidum, Geotrichum capitatum, Rhodotorula minuta* and *Rhodotorula rubra* solubilized insoluble phosphates. The isolated soil yeast solubilized insoluble phosphate in vitro leading to the formation of large amounts of soluble phosphate (Al-Fatih, 2005).

2.3. Statement of the Problem

Compared with the use of bacteria and mycorrihzal fungi, the use of yeasts as plant growth promoting agents has not been extensively investigated and as aresult data on the growth improvement of plants like rice, pepper, and tomato by yeasts are scarce or lacking. Most of the researchers have focused on the use of particular bacterial species, commonly referred to as plant growth promoting rhizobacteria (PGPR). The role of other microbial species, including yeasts, has received less attention (Tolba *et al*., 2016).

The chemical fertilizers used in the agriculture to improve plant growth and yield have a big harmful impact on ecosystem. Their overuse has hardened the soil, decreased soil fertility, polluted air and water, and released greenhouse gases, thereby bringing hazards to human health and environment as well. But plant growth promoting yeasts are not like them; they are environmentally friendly and slow release which will allow time for microbial activity to break down the organic materials in the yeasts (Ghoname *et al*., 2010).

The use of plant growth promoting yeasts is a better alternative to solve this problem. Thus, the present study was intended to isolate and identify yeasts from rhizospheric soil, tej ,tella, leaven, and Baker's yeast and to apply on Tomato, Rice, and Pepper seedlings cultivars using these yeast isolates. investigating the high potential yeast species that can promote the growth of those crop varieties under laboratory condition. Thus this study applies an eco-friendly treatments which is capable promote growth of crops within a short period of time with a low cost.

2.4. Significance of the Study

An increase in the human population has been witnessed during the past four decades concurrently increasing the demand of food. This increase in the demand for food has led to the need for improvements better plant nutrition to get higher yields. There are varieties of practices and improvements that could each contribute to increased efficiency. For example there are different types of yeast, plant growth promoting rhizobacteria (PGPR) and plant hormones that can enhance plant growth and development. They may enhance water-holding capacity, increase antioxidants, and enhance metabolism.

It is supposed that a better understanding of the role of soil yeasts in the plant growth promotion hold a key to future sustainable agricultural practices. Nowadays, a great attention has been focused on the possibility of using natural and safe agents for promoting growth of crops and for inducing its resistance against different diseases. Studies indicate that plant root growth may be directly or indirectly enhanced by yeasts in the rhizosphere(El-Bassiony *et al.*, 2014). A wide diversity of soil yeasts have been researched for their potential as bio-fertilizers. Yeast from different source is considered a new promising plant growth promoting yeast for different crops. It became in the last few decade a positive alternative to chemical fertilizers safely used for human, animal and environment. Due to its cytokinin content, yeast treatments were suggested to play a beneficial role in cell division and cell enlargement

2.5. Objectives

2.5.1. General objective

- The general objective of this study was to evaluate yeast isolates from different sources for their plant growth promoting ability for Rice (*Oryza sativa L.*), Pepper (*Capsicum annumL.*), and Tomato (*Lycopersicon esculentem L.*) seedlings.

2.5.2. Specific objectives

- To isolate, characterize and identify the plant growth- promoting of yeast isolates isolated from Tella, Tej, Leaven, rhizospheric soil and using baker's yeast collected from the local markets of Gondar town.
- To make a comparison among different yeast isolates for their plant growth- promoting performance.
- To investigate the treatment effect of yeast isolates on plant growth of Rice (*Oryza sativa L.*), Pepper (*Capsicum annumL.*), and Tomato (*Lycopersicon esculentem L.*) seedlings.

3. MATERIALS AND METHODS

3.1. Description of study area

The study was conducted in Microbiology laboratory (department of biology), University of Gondar which is situated in Gondar town, Ethiopia. Gondar town has a latitude and longitude of 12°36′N 37°28′E with an elevation of 2133 meters above sea level. Average rain fall is 57.25 mm/year, Average maximum and minimum temperature is 27ºC and, 15ºC respectively (Garedew *et al.,* 2014). Gondar has a humid subtropical mild summer climate that is mild with dry winters, mild rainy summers and moderate seasonality.

3.2. Study design

This study was performed by using cross sectional laboratory based experimental design. It was used complete random design techniques to collect experimental yeast samples (Tej, Tella, leaven, Baker's yeast and soil yeasts) from the local markets of Gondar town and from rhizospheric soil of the Biology department experimental farm site**.**

3.3. Collection of samples

Hundred ml of each Tej, Tella, leaven and Baker's yeast samples were collected randomly from the local markets of Gondar town using sterilized plastic bottles. Half kg of Soil sample from the rhizosphere of Maize were carefully removed from the farm land of University Gondar by digging up at the depth of 25cm using auger and bulked together from rhizospheric soil of UOG farm land and transferred using plastic bags. All the collected samples were brought to Microbiology Laboratory of department of Biology, University of Gondar and was kept in Refrigerator at temperature 4°C suitable and safe for the sample.

3.4. Isolation and identification of yeasts

3.4.1. Serial dilution

One g of soil sample was weighed out with sterile precaution and made into serial dilutions of (10^{-1}-10^{-6}) with sterile distilled water. The soil dilutions was shaken by using vortex mixer for few seconds, as each dilution made and was reshaken briefly before inocula was removed. Inoculums of 0.1ml was put in yeast peptone dextrose (YPD) agar plates using small glass pipette and it was spread throughly with a sterile bent glass rod. Plates were incubated upside

down at room temperature so as to prevent condensation from falling into the culture media and to prevent air borne contaminations.

One ml of tella, tej and leaven samples were mixed separately with 9ml sterile distilled water in a sterile flask. After that, one ml of the mixture was taken and serially diluted in test tubes each containing 9ml sterile distilled water. This is followed by spread plating aliquots of 0.1ml from appropriate dilutions (10^{-1}-10^{-6}) on YPD agar plates which was prepared in the presence of antibiotics (chloroamphenicol) to prevent the growth of other microorganisms other than yeasts (Pons et al., 1986). All the plates were incubated at 25ºC for 2 to 3 days. The colonies were transferred to slant YPDA cultures and preserved.

Representative colonies of all morphological types were randomly picked and subcultured on YPD agar media for purification. The purified cultures were maintained on YPD agar slant at 4°C and preserved in 80% glycerol at -22°C for subsequent experiments and all the cultures were checked for purity for each experiment

3.4.2. Isolation of active dry baker's yeast into pure culture

The active dry baker's yeast *Saccharomyces cerevisiae* by the name saf-levure, France was bought from a super market. By taking 0.5g of it suspended in sterile distilled water aseptically. Serial dilutions (10^{-1}-10^{-6}) were made to reduce the number of yeast cells as described above. Aliquots of 0.1ml of the suspensions were spread plated on YPDA. The cultures on the agar plate were incubated at 25ºC for 48hrs. The colonies were transferred to slant YPDA cultures and preserved at 4ºC for further study.

3.4.3. Pure culture methods

The pure yeast colonies appeared was further purified. Yeast colonies of different morphologies was selected and purified by cross-streaking on YPDA agar. Pure colonies were isolated and tested for further characterization.

3.4.4. Confirmation test

The samples of yeast isolates were grown on a differential medium, Potato Dextrose Agar (PDA) to confirm the isolations are really yeasts or not and was incubated at 28°C for 48 hours.

3.4.5. Identification of yeast isolates

Isolates of yeasts were identified through colonial and microscopic morphology, sugar assimilation and fermentation abilities, and some biochemical characteristics. all colonies were subjected to biochemical tests (based on the utilization of carbon and nitrogen sources).

3.5. Collection of plant seeds

Eight different crop seed varieties (3 rice, 3 tomato, and 2 pepper varieties) were collected from Adet agricultural research center, which is located in Adet town, West Gojjam zone and from Woreta Federal Rice production and Research Center, Woreta town, South Gondar using sterile plastic bags.

3.6. Methods of application of yeasts on seedlings of the test plant

The collected seed varieties were sown at department of Biology farm site, university of Gondar and it was grown for three weeks until their seedling stage. Consequently, the seedlings was transported into microbiology laboratory of department of biology, University of Gondar. Plant seedlings roots were washed free of soil, rinsed with tap water, and it was rinsed three times in calcium hypochlorite remove contamination from surfaces.

Around 150 (each pot was contained three seedlings) 4-L plastic pots were prepared and filled with 2.5 kg steam sterilized soil. Soil used for the whole experiment was taken from a single source. Uniform seedlings were immersed in four different yeast suspensions which were prepared by dissolving yeast colonies in distilled water and transplanted to 4-L plastic pots. The pot experiment was arranged in five treatments and in 3 replications. The control groups were transplanted without the supplementation of any yeast isolates. Plants were watered daily and grown for 6 weeks.

3.7. Assessment of vegetative plant growth

The growth patterns (stem elongation (height), growth of leaves, and no. of leaves, node, inter node and root colonization) of Tomato, Rice, and Pepper plant seedlings were examined,

investigated and measured four times between 10 days of measurement periods and the result was recorded and evaluated accordingly. Three plant seedlings from each replicated pots were randomly taken four times at every 10 days after sowing to evaluate the following vegetative growth characters: Stem height, number of node, number of internode, and number of leaves/plant. Shoot parts of Tomato, Pepper, and Rice (branches and leaves) were oven dried at 80^{o}C till constant weight. The dry weight of leaves and branches/ plant as well as whole plant was determined.

3.7.1. Root elongation bioassay

After 6 weeks of growth, uprooting the plant, the length and elongation of seedling roots were measured and compared with that of the roots of the untreated (controls) seedlings.

3.8. Data analysis

The data recorded was subjected to analysis using SPSS version 17 software. The data were also presented in descriptive statistics, graphs and diagrams using microsoft excel 2007. Statistical analysis was also undertaken by analysis of variance (ANOVA) .The results were considered statistically significant at P-value<0.05. Data are expressed as mean ± standard deviation (SD). The significance of differences between groups was determined using Student *t* tests and analyses of variance. $P<0.05$ were considered statistically significant. *$P<0.05$.

4. RESULTS

4.1 Morphological characterization of isolated yeasts

All the dominant yeasts isolated from the beverages and the ingredients did have round or oval shape and reproduced asexually by budding. Most of the isolates (I2, I3, andI4) that were isolated from Tella, Tej, and Leaven were flat, smooth, moist, glistening or dull, and cream in color. They reproduced sexually by forming round ascospores in which their asci contained one up to four ascospores. All the isolated yeasts also formed pseudo hyphae when they were inoculated on corn meal agar, nitrogen deficient media. I5 which was *isolated* from rhizospheric soil were appeared as large, round, white or cream colonies.

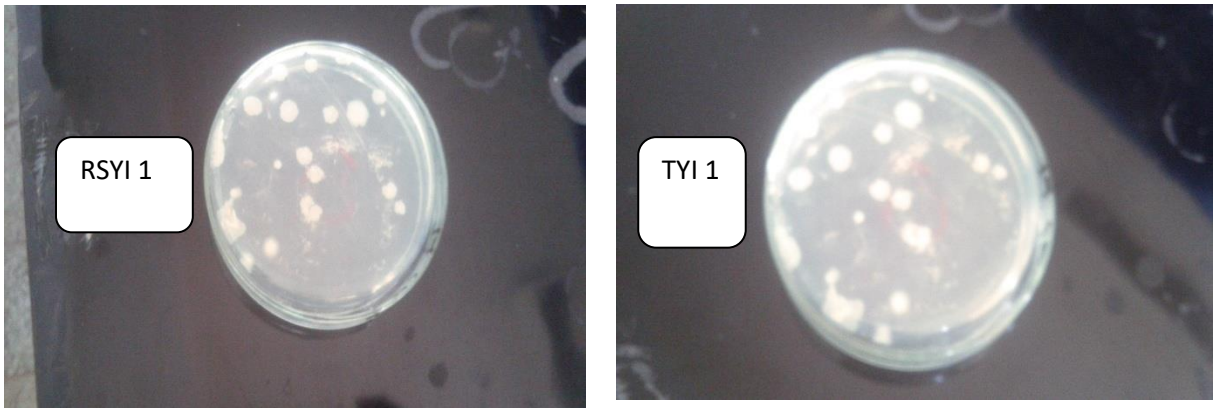

Figure 1 Morphological observation of some isolates, (RSYI 1=Rhizospheric soil yeast isolate 1, TYI 1=, Tella yeast isolate 1).

Table 1.Morphological characteristics observed in four isolates after culturing on PDA agar for 2-3 days at 28^{o} in an aerobic growth chamber

Yeast isolates	Shape	Color	Surface	Elevation	Margin
I1	Spherical	White/cream	Smooth	Convex	Entire
I2	Spherical/ Oval	Whitish	Smooth	Convex	Regular edge
I3	Ellipsoid	White	Wrinkled	Convex	Entire
I4	Round	White/cream	Smooth	Convex	Entire
I5	Spherical/ Ovoid	Blue-grey	Smooth	Slightly/ convex	Undulating

Note: (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil).

4.1.2 Physiological tests of the isolated yeasts

The Physiological properties of the dominant yeast isolates of the sources and the beverages during fermentation test are shown in Table 2. All of them were found to be positive for the fermentation of glucose, sucrose, galactose, maltose, and fructose; negative for lactose, Dextrose and mannitol. The results of the carbohydrate fermentation tests and morphological observation comparing with the standard keys of yeasts (Barnett *et al*., 200) showed that the dominant yeasts isolated from the beverages and the ingredients were all I2, I3 and I4 belong to the genus Saccharomyces most of them are *Saccharomyces cervisiae.* I5 belong to the genus *Candida* most probably *Candida tropicalis.* Below (Table 2) show the carbohydrate fermentation characteristics of the isolated yeasts. As presented in the table, most of the isolates were fermentative for Glucose, Sucrose, Fructose, Maltose, and Galactose. They are non fermentative for Lactose, Dextrose, and Mannitol carbohydrates.

Table 2. Carbohydrate fermentation test of different yeast isolates (+ = Positive,−= Negative)

yeast isolates	Glucose	Sucrose	Fructose	Maltose	Galactose	Lactose	Dextrose	Mannitol
I1	+	+	+	+	+	-	-	-
I2	+	+	+	+	+	-	-	-
I3	+	+	+	+	+	-	-	-
I4	+	+	+	+	+	-	-	-
I5	+	+	+	+	+	-	-	-

Note: (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil).

4.2. Effect of yeast isolates on the growth characters of Pepper, Rice, and Tomato seedlings Cultivars

4.2.1. Effect of isolated yeasts on Pepper plant Seedlings cultivars

Table 3 shows the effect of different yeast isolates on the growth characters (Stem elongation, number of nodes, number of inter nodes, and number of leaf branches, root length, fresh and dry weight) of pepper Indiyam (IM) 42. All the treatments have a significant variation from the control (p=0.05), but there is no significance between treatments. Rhizospheric soil yeast isolate (I5) gives the highest growth in stem length (15.05 cm), number of nodes (9.75), number of internodes (8.75), number of leave branches (10.75). root length (13.1 cm), fresh weight (15.2 g) and dry weight (3.5 g) and On the other hand, the lowest significant values were obtained with corresponding isolates from leaven (I4) with stem length (14.1 cm), number of nodes (7.75), number of internodes (7.35), number of leave branches (9.65), root length (10.6 cm), fresh weight (6.6 g) and dry weight (1.1 g). As indicated by the following data table (3), treatment of pepper IM 42 with I1, I2, I3, I4 and I5 gave similar and significant values compared to control groups. Isolate 5 which were isolated from the rhizospherc soil showed the highest values in all growth parameters followed by Baker's yeast, Isolate 2 (I2), Isolate 3 (I3), and Isolate 4 (I4) respectively compared to the control groups. Pepper IM 42 was effectively improved by the treatments compared to pepper markofana.

Table 3 Effect of yeast isolates on the growth parameters of Pepper INDIYAM (IM) 42

Yeast isolates	Stem length(cm)	No of nodes	No of Inter nodes	No of leave branches	Root length(cm)	Fresh weight(g)	Dry weight(g)
I1	14.85±0.12*	9.25±0.12*	8.25±0.12*	10.55±0.12*	11±0.12*	14.5±0.12*	3.25±0.12*
I2	14.55±0.12*	8.35±0.12*	7.85±0.12*	10.05±0.12*	11.1±0.12*	12.3±0.12*	1.7±0.12*
I3	14.35±0.12*	8.05±0.12*	7.55±0.12*	9.85±0.12*	10.8±0.12*	12±0.12*	1.6±0.12*
I4	14.15±0,12*	7.75±0.12*	7.35±0.12*	9.65±0.12*	10.6±0.12*	6.6±0.12*	1.1±.0.12*
I5	15.05±0.12*	9.75±0.12*	8.75±0.12*	10.75±0.12*	13.1±0.12*	15.2±0.12*	3.5±0.12*
Control	10.65±0.12	6.05±0.12	5.05±0.12	5.35±0.12	5.6±0.12	2.5±0.12	0.3±0.03
L.S.D at 5 %	3.5	1.7	2.3	4.3	5.0	4.1	0.766

a Values with asterisks(*) within a column are not significantly different as determined by the LSD test (P 0.05).
b. (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil).
c. Control: seedlings without yeast treatment

Data presented in Table 4 indicated that treatment of Pepper Markofana variety with isolated yeasts improved plant growth character as compared to untreated plants. The highest significant values were achieved by rhizospheric yeast isolate (I5) with values of stem length (14.55 cm), number of nodes (9.85), number of internodes (8.85), number of leave branches (10.75), root length (11.8 cm), fresh weight (11.5 g) and dry weight (1.8 g). The corresponding lowest values were obtained by yeasts isolated from leaven (I4); Stem length (13.65 cm), number of nodes (6.35), number of internodes (5.75), number of leave branches (9.35), root length (8.5 cm), fresh weight (4.7 g), and dry weight (0.5 g). These findings were significant and true in all treatments of yeasts.

Table 4 Effect of yeast isolates on the growth parameters of Pepper Markofana

Yeast isolates	Stem length(cm)	No of nodes	No of Inter nodes	No of leave branches	Root length(cm)	Fresh weight(g)	Dry weight(g)
I1	14.35±0.12*	8.05±0.12*	7.05±0.12*	10.35±0.12*	10.8±0.12*	9.3±0.12*	1.6±0.12*
I2	14.05±0.12*	7.35±0.12*	6.55±0.12*	10.05±0.12*	10.1±0.12*	8.7±0.12*	1.4±0.12*
I3	13.85±0.12*	6.85±0.12*	6.35±0.12*	9.85±0.12*	9.2±0.12*	7.03±0.12*	1.3±0.12*
I4	13.65±0.12*	6.35±0.12*	5.75±0.12*	9.35±0.12*	8.5±0.12*	4.7±0.12*	0.5±0.12*
I5	14.55±0.12*	9.85±0.12*	8.85±0.12*	10.75±0.12*	11.8±0.12*	11.5±0.12*	1.8±0.12*
Control	8.25±0.12	5.95±0.12	5.35±0.12	6.05±0.12	5.3±0.12	2±0.12	0.1±0.12
L.S.D at 5 %	5.4	0.4	0.4	3.3	3.2	2.7	0.366

a Values with asterisks(*) within a column are not significantly different as determined by the LSD test (P 0.05).
b. (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil).
c. Control: seedlings without yeast treatment

4.2.2. Effect of application of isolated yeasts on Rice seedlings

Data presented in Table (5) reveal the treatment effect of yeast isolates on the stem length, number of leave branches as well as fresh weight and dry weight measurements of Rice X-Jigna Cultivar. As shown in the table, yeasts isolated from the rhizospheric soil significantly increased all the growth parameters followed by Baker's yeast, I2, I3, and I4 respectively compared to the control treatments, which were not treated with any yeast isolates.

The highest values of Rice X-jigna seedling variety were achieved by I5 with Stem length (14.45 cm), number of nodes (8.75), number of internodes (8.05), number of leave branches (11.05) root length (12.3 cm), fresh weight (11 g), dry weight (2.6 g). and the corresponding lowest value Leaven yeast isolate (I4) were Stem length (13.55 cm), number of nodes (6.45), number of internodes (6.55), number of leave branches (9.35) root length (9.9 cm), fresh weight (5.2 g), dry

weight (0.7 g). There is a significant variation between treatments and the control groups (α=0.05), but the variation is not significant between treatments.

Table 5 Effect of yeasts on growth parameters of Rice X-jigna

Yeast isolates	Stem length(cm)	No of leaves	No of inter nodes	No of nodes	Root length(cm)	Fresh weight(g)	Dry weight(g)
I1	14.25±0.13*	10.75±0.13*	7.35±0.13*	8.45±0.12*	11±0.13*	9.6±0.12*	1.25±0.12*
I2	13.95±0.13*	10.35±0.13*	7.15±0.13*	7.75±0.12*	11.4±0.13*	5.5±0.12*	1.3±0.12*
I3	13.75±0.13*	9.75±0.12*	6.75±0.12*	7.05±0.12*	10.1±0.12*	5.4±0.12*	0.8±0.12*
I4	13.55±0.13*	9.35±0.12*	6.55±0.12*	6.45±0.12*	9.9±0.12*	5.2±0.12*	0.7±0.12*
I5	14.45±0.13*	11.05±0.13*	8.05±0.13*	8.75±0.12*	12.3±0.12*	11±0.12*	2.6±0.12*
Control	8.05±0.12	5.25±0.12	5.75±0.12	5.75±0.12	6.1±0.12	1.3±0.12	0.2±0.12
L.S.D at 5 %	5.5	4.1	0.8	0.7	3.8	3.9	0.5

a Values with asterisks(*) within a column are not significantly different as determined by the LSD test (P 0.05).
b. (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil). c. Control: seedlings without yeast treatment

Data in Table 6 show the effect of treatment of yeast isolates on growth characters of Rice ediget cultivar. I5 (Rhizospheric soil isolate) achieved the highest measurement of stem length (14.85 cm), number of nodes (8.25), number of internodes (8.25), number of leave branches (11.35) root length (11.6 cm), fresh weight (11.2 g), dry weight (2.8 g) followed by Baker's yeast compared to the untreated (control) groups. The lowest values of Rice ediget growth parameters were recorded in leaven yeast isolate (I4) with values of stem length (13.95 cm), number of nodes (6.75), number of internodes (6.75), number of leave branches (9.55) root length (8.7 cm), fresh weight (4.8 g), dry weight (0.8 g).

Table 6 Effect of yeasts on growth parameters of Rice ediget

Yeast isolates	Stem length(cm)	No of leaves	No of inter nodes	No of nodes	Root length(cm)	Fresh weight(g)	Dry weight(g)
I1	14.55±0.28*	10.75±0.13*	7.75±0.12*	8.75±0.10*	11.3±5.3*	11±9.5*	2.25±2.0*
I2	14.25±0.08*	10.35±0.13*	7.25±0.12*	8.05±0.1*	10.6±0.12*	8.3±0.12*	1.8±0.12*
I3	14.15±0.05*	10.05±0.13*	7.05±0.12*	7.35±0.12*	9.9±0.12*	5.5±0.12*	0.9±0.1*
I4	13.95±0.01*	9.55±0.13*	6.75±0.12*	6.75±0.12*	8.7±0.12*	4.8±0.12*	0.8±0.12*
I5	14.85±0.05*	11.35±0.13*	8.25±0.12*	9.05±0.1*	11.6±0.12*	11.2±0.12*	2.8±0.12*
Control	8.85±0.12	5.35±0.13	5.05±0.12	6.05±0.12	6±0.12	1.5±0.12	0.2±0.03
L.S.D at 5 %	5.1	4.2	1.7	0.7	2.7	3.3	0.566

a Values with asterisks(*) within a column are not significantly different as determined by the LSD test (P 0.05).
b. (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil). c. Control: seedlings without yeast treatment

Data in Table (7) show clearly that, application of isolated yeasts significantly increased Stem length, Number of leaves, number of nodes and internode, root elongation, and fresh and dry weight of Rice Getachew cultivar compared to untreated groups. The highest values were obtained from I5 (rhizospherc soil isolate) with Stem length (14.05 cm), number of nodes (8.45), number of internodes (7.75), number of leave branches (10.75). Root length (12.7 cm), fresh weight (10.5 g), dry weight (1.5 g) and the lowest values were from I4 (isolates from leaven) With Stem length (13.05 cm), number of nodes (6.15), number of internodes (6.35), and number of leave branches (9.35). Root length (8.7 cm), fresh weight (6.4 g), dry weight (0.5 g).

Table 7 Effect of yeasts on growth parameters of Rice Getachew variety

Yeast isolates	Stem length(cm)	No of leaves	No of inter nodes	No of nodes	Root length(cm)	Fresh weight(g)	Dry weight(g)
I1	13.75±0.12*	10.35±0.1*	7.45±0.12*	8.15±0.29*	12.5±0.12*	8.8±0.12*	1.15±0.12*
I2	13.45±0.12*	10.05±0.1*	6.35±0.12*	7.45±0.04*	10.3±0.12*	6.9±0.12*	0.7±0.12*
I3	13.25±0.12*	9.75±0.1*	7.05±0.12*	6.75±0.13*	9.9±0.12*	7±0.12*	0.7±0.12*
I4	13.05±0.12*	9.35±0.1*	6.35±0.12*	6.15±0.13*	8.7±0.12*	6.4±0.12*	0.5±0.12*
I5	14.05±0.12*	10.75±0.1*	7.75±0.12*	8.45±0.08*	12.7±0.12*	10.5±0.12*	1.5±0.12*
Control	7.85±0.12	5.35±0.1	4.75±0.12	5.45±0.12	5.1±0.12	0.8±0.12	0.1±0.03
L.S.D at 5 %	5.2	4.0	1.6	0.7	3.6	5.6	0.366

a Values with asterisks(*) within a column are not significantly different as determined by the LSD test (P 0.05).
b. . (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil).
c. Control: seedlings without yeast treatment

4.2.3. Effect of application of isolated yeasts on Tomato seedlings

Similar to those of pepper varieties, the growth of tomato varieties were also effectively improved and stimulated by the application of the isolated yeasts. Table (8) shows the treatment effect of different yeast isolates on the growth parameters of tomato melksalsa seedling variety. Yeasts isolated from the rhizospheric soil yeast (I5) showed the highest positive effect on the growth of tomato melksalsa seedlings with stem length (17.45 cm), number of nodes (9.35), number of internodes (9.05), number of leave branches (13.35) root length (14.5 cm), fresh weight (13.8 g), dry weight (2.3 g).

While Leven yeast isolate (I4) were achieved the lowest significant values with stem length (16.25 cm), number of nodes (8.05), number of internodes (7.35), number of leave branches (11.55) root length (11.1 cm), fresh weight (11.16 g), dry weight (0.7 g), other yeast isolates were also significantly improved the growth character of Tomato melksalsa variety.

Table 8. Effect of yeasts on growth parameters of Tomato melksalsa variety

Yeast isolates	Stem length(cm)	No of leaves	No of inter nodes	No of nodes	Root length(cm)	Fresh weight(g)	Dry weight(g)
I1	17.15±0.12*	13.05±0.12*	8.65±0.12*	9.05±0.12*	13.7±0.12*	13.6±0.12*	1.45±0.12*
I2	16.85±0.12*	12.65±0.12*	8.25±0.12*	8.75±0.12*	12.8±0.12*	12.8±0.12*	1.3±0.12*
I3	16.45±0.12*	12.05±0.12*	7.55±0.12*	8.35±0.12*	12.5±0.12*	12.5±0.12*	1.2±.0.12*
I4	16.25±0.12*	11.55±0.12*	7.35±0.12*	8.05±0.12*	11.1±0.12*	11.16±0.12*	0.7±.0.12
I5	17.45±0.12*	13.35±0.12*	9.05±0.12*	9.35±0.12*	14.5±0.12*	13.8±0.12*	2.1±0..12*
Control	10.25±0.12	7.35±0.12	6.05±0.12	7.05±0.12	6.5±0.12	6.3±0.12	0.5±0.12
L.S.D at 5 %	6.0	4.2	1.3	1.6	4.6	4.8	0.2

a Values with asterisks(*) within a column are not significantly different as determined by the LSD test (P 0.05).
b. . (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil).
c. Control: seedlings without yeast treatment

Data in table (9) below reveal that highest significant values of tomato kochero in stem length (16.2 cm), number of nodes (9.05), number of internodes (8.75), and number of leave branches (13.05) root length (14.1 cm), fresh weight (13.6 g), and dry weight (2.5 g) were achieved by the treatment of rhizospheric yeast isolate (I5).

The lowest significant values were obtained in I4 with values of stem length (15.1 cm), number of nodes (7.75), number of internodes (7), number of leave branches (11.25) root length (10.1cm), fresh weight (11 g), dry weight (1.1 g). All other treatments (I1, I2and I3) were also significantly improved the growth parameters of tomato kochero compared to the control groups.

Table 9 Effect of yeasts on growth parameters of Tomato kochero variety

Yeast isolates	Stem length(cm)	No of leaves	No of inter nodes	No of nodes	Root length(cm)	Fresh weight(g)	Dry weight(g)
I1	15.9±0.12*	12.75±0.12*	8.35±0.12*	8.75±0.12	13±0.12*	13.1±0.12*	1.85±0.12*
I2	15.6±0.12*	12.35±0.12*	7.95±0.12*	8.45±.0.12*	12.8±0.12*	11.8±0.12*	1.7±0.12*
I3	15.3±0.12*	11.75±0.12*	7.25±0.12*	8.05±0.12*	12.3±0.12*	11.6±0.12*	1.5±0.12*
I4	15.1±0.12*	11.25±0.12*	7.05±0.12*	7.75±0.12*	10.1±0.12*	11±0.12*	1.1±.0.12*
I5	16.2±0.12*	13.05±0.12*	8.75±0.12*	9.05±0.12*	14.1±0.12*	13.6±0.12*	2.5±0.12*
Control	10.0±0.12	7.05±0.12	5.75±0.12	6.75±0.12	6±0.12	5.2±0.12	0.3±0.05
L.S.D at 5 %	5.1	4.2	1.3	1.0	4.1	5.8	0.8

a Values with asterisks(*) within a column are not significantly different as determined by the LSD test (P 0.05).
b. (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil). c. Control: seedlings without yeast treatment

Data in Table (10) reports the effect of application of isolated yeasts on the growth characters of Tomato Miya seedling variety. As shown in the table the highest stem length (15.85 cm), number of nodes (8.75), number of internodes (8.45), and number of leave branches (12.75), root length (13.1 cm), fresh weight (13.3 g), and dry weight (2.7 g) were achieved by rhizospheric yeast treatment (I5). While the lowest measurements were obtained by leaven yeast isolates that is Stem length (14.95 cm), number of nodes (7.45), number of internodes (6.75), and number of leave branches (10.95), root length (11 cm), fresh weight (8.5 g), and dry weight (1.7 g).

Table 10 Effect of yeasts on growth parameters of Tomato Miya variety

Yeast isolates	Stem length(cm)	No of leaves	No of inter nodes	No of nodes	Root length(cm)	Fresh weight(g)	Dry weight(g)
I1	15.55±0.12*	12.45±0.12*	8.05±0.12*	8.45±0.12*	12.1±0.12*	13.1±0.12*	3±0.12*
I2	15.25±0.12*	12.05±0.12*	7.65±0.12*	8.15±0.12*	11.8±0.12*	11.5±0.12*	1.4±0.12*
I3	15.15±0.12*	11.45±0.12*	6.95±0.12*	7.75±0.12*	11.1±0.12*	9.9±0.12*	0.6±0.12*
I4	14.95±0.12*	10.95±0.12*	6.75±0.12*	7.45±0.12*	11±0.12*	8.5±0.12*	1.7±0.12*
I5	15.85±0.12*	12.75±0.12*	8.45±0.12*	8.75±0.12*	13.1±0.12*	13.3±0.12*	2.7±0.12*
Control	9.85±0.12	6.75±0.12	5.45±0.12	6.45±0.12	4±0.12	5±0.12	0.2±0.05
L.S.D at 5 %	5.1	4.2	1.3	1.0	7.0	3.5	0.4

a Values with asterisks(*) within a column are not significantly different as determined by the LSD test (P 0.05).
b. (I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil).
c. Control: seedlings without yeast treatment

Figure (1) gives an example of the positive effect of the isolated rhizospheric soil yeasts on Pepper Markofana growth characters. As illustrated in the figure, the Growth of the treated (right side) Pepper Markofana variety were increased due to the application of yeasts isolated from rhizospheric soil compared to the control treatments. Similarly other growth parameters (number of leaves, stem height, root length, fresh weight, dry weight, number of node and number of internode) were also increased because of the application of yeasts. As we can see from the figure Rhizospheric soil yeast treatment were showed the highest values in all growth parameters of Pepper markofana.

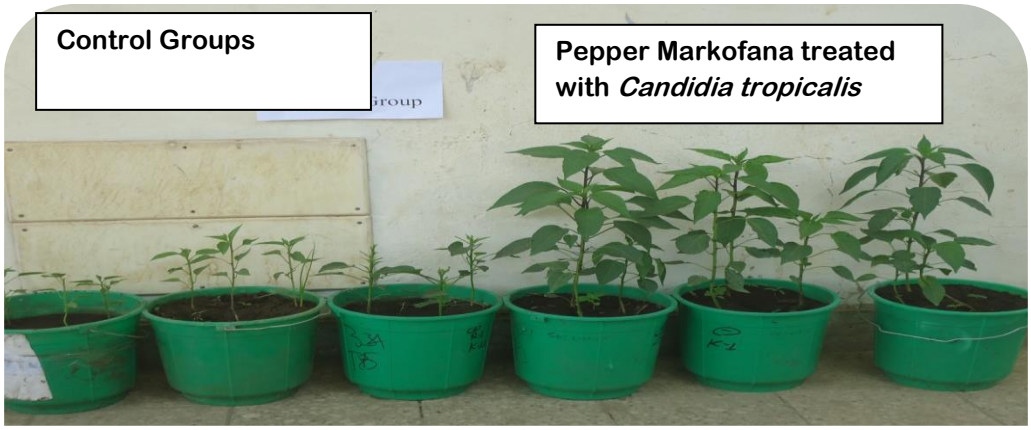

Figure 2.Effect of rhizospheric soil yeast on some plant growth characters Pepper Markofana seedling varieties

The treatment effect of rhizospheric soil yeasts on the growth of Rice X-jigna seedlings illustrated in the figure (2). The growth character of the treated (right side) Rice X-jigna variety were increased due to the application of yeasts isolated from rhizospheric soil (I4) compared to the control treatments.

Similarly other growth parameters (number of leaves, stem height, root length, fresh weight, dry weight, number of node and number of internode) were also increased in Rice x-jigna as a result of the application of yeasts. As we can see from the figure rhizospheric soil yeast treatment were showed the highest values in all growth parameters of Rice X-jigna. The number of tillers was also significantly higher in the treated rice seedlings compared to control groups

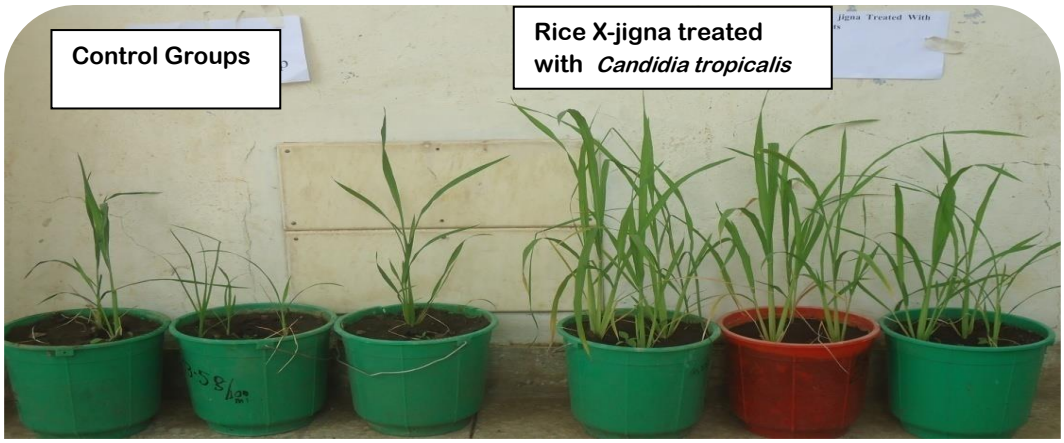

Figure 3. Growth performance of Rhizospheric soil yeast on Rice X- jigna seedling varieties

Figure 3. Presents the treatment effect of Baker's yeast (*saccharomyces cervisae*) on the growth of tomato Kochero seedlings. As shown in the figure, the shoot height of the *S.cerevisae* treated tomato kochero seedlings is significantly greater than the shoot height of those control groups. Other growth characters of tomato seedlings were also showed the same positive result.

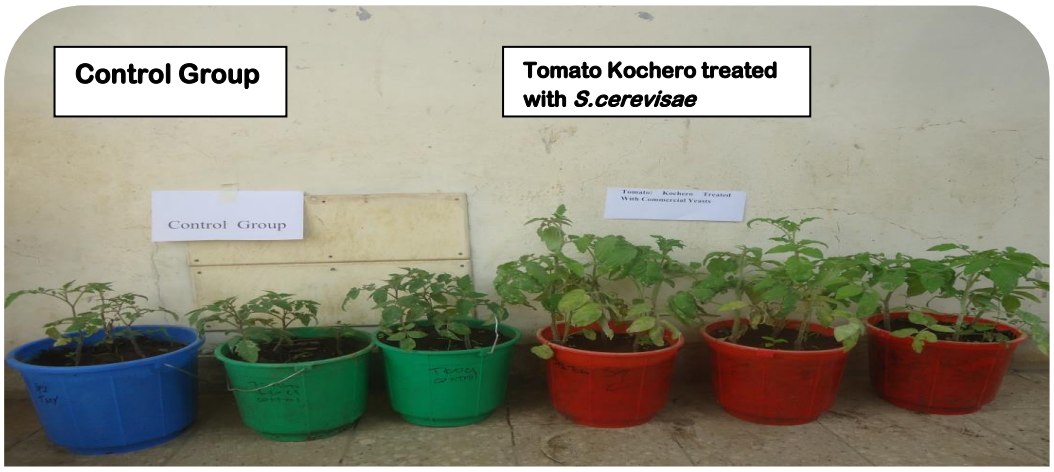

Figure 4. Effect of Baker's yeast on the growth of Tomato Kochero seedling varieties

4.3. Efficacy of plant growth promoting yeasts

The effectiveness of plant growth promoting yeasts (PGPY) in Tomato, Pepper, and Rice seedlings were clearly shown in this finding. *Candidia tropicalis* isolated from the rhizosphere of Maize effectively enhanced the growth and development of the test plants. *Saccharomyces cerevisae* obtained from Baker's yeast were ranked second in its effectiveness against the growth

of those seedling varieties. Other yeast isolates (from Tella, Tej, and leaven) were also improved the growth of Tomato, Pepper, and Rice seedling cultivars.

Data in Table (11) show root elongation effectiveness tests of Tomato, Pepper, and Rice seedling varieties. Regarding the effect of yeasts on the root length, the highest significant values were achieved by treatment with rhizospheric soil yeast (I5) is highly effective Which are: Pepper IM 42 (57.25%), Pepper Markofana (55.08%), Rice x-jigna (50.40%), Rice ediget (48.275%), Rice Getachew (59.84%), Tomato melksalsa (55.17%), Tomato kochero (57.44%) and Tomato miya (69.46%) and the corresponding lowest values obtained by Leaven yeast Isolates (I4) are Pepper IM 42 (47.17 %), Pepper Markofana (37.64%), Rice x-jigna (38.38%), Rice ediget (31.03%), Rice Getachew (41.3%), Tomato melksalsa (41.44%), Tomato kochero (41.59%) and Tomato miya (63.63%)

Table 11 Efficacy of isolated yeasts in increasing the root length of Tomato, Pepper, and Rice varieties

Root elongation efficacy test (%)

Yeast Isolates	Pepper IM 42	Pepper Markofana	Rice x-jigna	Rice ediget	Rice Getachew	Tomato melksalsa	Tomato kochero	Tomato miya
I1	49.09	50.92	44.54	46.90	59.2	52.55	53.84	66.94
I2	49.54	47.52	46.49	43.39	50.48	49.21	53.12	66.10
I3	48.15	42.39	39.60	39.39	48.48	48	51.21	63.96
I4	47.17	37.64	38.38	31.03	41.3	41.4	40.59	63.63
I5	57.25	55.08	50.40	48.27	59.84	55.17	57.44	69.46
L.S.D at 5 %	5.0	3.2	3.8	2.7	3.6	4.6	0.7	7.00

Note :(I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil)

Data in Table (12) present the shoot fresh weight efficacy of different yeast isolates (I1, I2, I3, I4, I5,) of Tomato, Pepper, and Rice seedling varieties. As stated in the table, the treatment effect of yeast isolates significantly increased the percent of shoot fresh weight of plants. The highest values recorded in rhizospheric soil yeast isolate (I5) are Pepper IM 42 (83.55%), Pepper Markofana (82.6%), Rice x-jigna (88.18%), Rice ediget (86.6%), Rice Getachew (92.38%), Tomato melksalsa (54.34%), Tomato kochero (61.76%) and Tomato miya (62.4%). While the lowest corresponding values were obtained as a result of application of leaven yeast isolates (I4)

Pepper IM 42 (62.12 %), Pepper Markofana (57.44%), Rice x-jigna (75%), Rice ediget (68.75%), Rice Getachew (87.5%), Tomato melksalsa (43.54%), Tomato kochero (52.72%) and Tomato miya (41.17%)

Table 12 Efficacy of isolated yeasts in increasing the root length of Tomato, Pepper, and Rice varieties

Shoot fresh weight efficacy test (%)

Yeast Isolates	Pepper IM 42	Pepper Markofana	Rice x-jigna	Rice ediget	Rice Getachew	Tomato melksalsa	Tomato kochero	Tomato miya
I1	82.75	78.49	86.45	86.36	90.90	53.67	60.30	61.83
I2	79.67	77.01	76.36	81.92	88.40	50.78	55.93	56.5
I3	79.16	71.55	75.92	72.72	88.57	49.6	55.17	49.49
I4	62.12	57.44	75	68.75	87.5	43.54	52.72	41.17
I5	83.55	82.60	88.18	86.60	92.38	54.34	61.76	62.40
L.S.D at 5 %	4.1	2.7	3.9	3.3	5.6	4.9	0.5	3.5

Note :(I1= Isolate 1 from Baker's yeast, I2=Isolate 2 from Tella, I3=Isolate 3 from Tej, I4= Isolate 4 from Leaven, I 5= Isolate 5 from Rhizospheric soil)

5. DISCUSSION

Data in (Table 3) showed that all parameters of Tomato, Pepper, and Rice in terms of growth significantly affected by application methods of yeast. Rhizospheric soil (15.05 cm stem height, 13.1 cm root length 13.2 g fresh weight, and3.5g dry weight) yeast treatment recorded the positive and significant superiority for most growth parameters of Pepper IM 42 varieties. Such result is in agreement with Amprayn *et al.(* 2012) which indicates that the inclusion of soil yeast CtHY in the commercial biofertiliser product BioGro, demonstrated to increase the nutrition, growth and yield of paddy rice. Number of node and Inter node and number of leave branches measurement also showed positive responses to the applied treatments.

Table (4) shows the application of yeast isolates on the growth parameters of Pepper Markofana significantly enhanced the length of stem (14.55cm), number of nodes (9.85) and internodes (8.85), number of leave branches (10.75), root length (11.8 cm), fresh (11.5 g) and dry (1.8g) weight under the treatment of rhisospheric yeast isolate (I5), which showed the highest treatment effect on Pepper Markofana seedlings. These results agree with other studies which have done in Cucumber Plants (Shehata *et al.*, 2012). Other investigators found similar results on the stimulatory effects of yeasts on plants such as on potato (Ahmed *et al*., 2011), Grapevines (Fawzi *et al*., 2014) and on sugar beet (Nemeat *et al*., 2015).

Yeast application significantly affected the values of growth parameters in all four measurement periods. Stem length (14.45 cm), fresh weight (11 g) and dry weight (2.6 g) of rice X-jigna varieties recorded the highest significant values under treatments Rhizospheric soil yeast isolate treatments followed by Baker's yeast which were 14.25 cm stem length, 9.6 g fresh weight, 1.25 g dry weight and 9.9 cm root length compared to control treatments (Table 5). Number of node and inter node measurements were also showed positive responses to the applied treatments. Rhizospherc soil yeast was found to be persistently stimulated both Tomato Kochero and Tomato Miya varieties compared to other treatments and the control groups.

The results obtained in this study showed that treatment of plants by yeast markedly increased all growth parameters including plant height, number of leaves, and number of branches and fresh weight of plants. The treatments also significantly enhanced Tomato, Pepper, and Rice root colonization as length of root elongations were significantly increased in response to the application of the treatments. This is in harmony with El-Bassiony *et al.* (2014) which pointed

out that yeast treatments showed the highest values of cytokinins, IAA and GA3 of leaves. Data in this result indicates that the Baker's yeast, *Saccharomaces cervisae* were shown to significantly improve the growth of *Tomato*, Pepper, and Rice seedlings. Such findings were confirmed in maize plants (Tolba *et al*., 2016). Nour and Tolba. (2015) which indicates that *Saccharomyces cerevisae* significantly enhanced plant height, number of branches, and shoot fresh and dry weights of plant during vegetative stages of chamomile plants. Moreover, *Saccharomyces cerevisae* has a regulatory role in promoting productivity of many plants such as sugar beet plants (El-Nady and Shalaby, 2008): Potato cv. Desiree **(** Sarhan and Abdullah, 2010). The common Bakery's yeast, *Saccharomyces cerevisae*, is known to improve plant growth and development due to its effects on cell division and differentiation.

In this study, five tested microorganisms, (Baker's yeast, Tella yeasts, Tej yeasts, Leaven dough yeasts, and rhizospheric soil yeasts) were investigated for their role in plant growth. As shown in the results the growth of Tomato, Pepper, and Rice transplanted and grown on pots when treated with yeasts gave higher growth and development in comparison with control treatments. Foliar application with yeast and chitosan increased significantly the vegetative growth, yield and its quality of cucumber. Meanwhile, foliar spraying with active dry yeast at rates of 4 g L-1 recorded highest values of T.S.S., N (%), Fe, Zn, Cu and Mn (mg kg-1) in cucumber fruits (Shehata *et al* 2012). The results in Table (6) also indicated that the highest values of fresh weight (11.2 g), dry weight (2.8 g) and numbers of node (8.25) and internode (8.25) in Rice ediget growth of plant shoots were obtained by the application of yeasts isolated from rhizospheric soil and the lowest values were obtained fresh weight (4.8 g), dry weight (0.8 g) and numbers of node (6.75) and internode (6.75) under Isolate 4 (isolates of leaven) compared to other treatments and controls. These findings are in agreement with the results of El-Tarabily and Sivasithamparam. (2006) indicated that the potential of soil yeasts to suppress a wider range of soil-borne fungal plant pathogens and to promote plant growth.

Data in Table (7) demonstrated that, using yeasts as a foliar application gave the best growth characters of rice getachew The highest values were obtained from I5 (rhizospherc soil isolate) with Stem length (14.05 cm), number of nodes (8.45), number of internodes (7.75), number of leave branches (10.75). Root length (12.7 cm), fresh weight (10.5 g), dry weight (1.5 g).

Meanwhile, the lowest values were found by I4 (isolates from leaven) with Stem length (13.05 cm), number of nodes (6.15), number of internodes (6.35), and number of leave branches (9.35). Root length (8.7 cm), fresh weight (6.4 g) and dry weight (0.5 g). These results were in agreement with those obtained from EL-Yazied and Mady. (2012) on broad bean and Asma *et al.* 2013 on the Pea Plant Growth.

Rhizospheric soil yeast isolates treatments in Table (8) showed the highest values of stem length (17.45 cm), number of node (9.35) and inter node (9.05), fresh weight (13.8 g), dry weight (2.1 g), root length (14.1 cm) and number of leave branches (13.35) in Tomato melksalsa compared to other treatments. This finding is in harmony with Hussain and Khalaf, (2007) found that spraying yeast solution treatments significantly increased plant height, number of branches/plant, dry matter of vegetative growth, number of tubers/plant, dry matter percentage of tubers, yield/plant, dry matter percentage of tuber, yield/plant and TSS.

Data in Table (9) indicate that all the applied yeast isolates significantly increased plant height, number of branches and leaves/plant and fresh and dry weight/plant, as well as number of node and internode of tomato kochero seedling varieties compared with control (untreated seedlings). The tallest plants with more branches (16.2 cm) and leaves (13.05) and heaviest fresh (13.6 g) and dry weight (2.5 g) of plant were obtained by treatment of rhizospheric yeast isolate (I5). These results were inconformity with those obtained by El-Tohamy and El-Greadly, 2007; Shokr and Fathy, 2009; and Nour *et al*., 2012 on snap bean plants, Ahmed, 2005; on pea plants.

The results presented in the Table (10) clearly revealed that, yeast treatment had significant effect on all growth characteristics (Stem length, number of node, number of internode, fresh weight, dry weight, root elongation and number of leave branches) of tomato miya seedling variety. Treatments I2, I3, and I4 gave similar results. There is a significant variance from the control (α=0.05), but between treatments it is not significant. Treatment with (I5 and I1) gives the largest growth effect, followed by the treatment with (I2, I3, and I4). The results are in agreement with those obtained by Ahmed *et al*., (1989) and Darwish & Ahmed (1993).

Similarly, New *et al*. (2013) indicated that Treatment of salt affected soils by these isolates; some mineral contents in soils were increased. Growths of maize in treated soils were higher than those of untreated soils. The highest values of Stem length measurements were obtained by the application of Rhizospheric soil followed by Baker's yeast, Tella yeast, Tej yeast, and Leaven yeast respectively. Those effects were consistent on all measured vegetative parameters such as plant height, number of leaves, number of branches and fresh and dry weights. These results are in harmony with the findings of El-Tohamy *et al*. (2008) who reported that foliar application of yeasts resulted in a significant increment of vegetative growth (including plant height, number of leaves, number of branches and fresh weight of plants) and yield of eggplant compared to control plants.

The efficacy root elongation in Table (11) by yeast isolate 5 were achieved the highest percentage in Pepper IM 42 (57.25%), Pepper Markofana (55.08%), Rice x-jigna (50.40%), Rice ediget (48.275%), Rice Getachew (59.84%), Tomato melksalsa (55.17%), Tomato kochero (57.44%) and Tomato miya (69.46%). Moreover, Gomaa *et al*. (2005) reported that inclusion the foliar application of yeast to the organic fertilization significantly increased potato yield in comparison with either the positive control or the corresponding treatments. Likewise, El-Tohamy *et al*. (2008) reported that foliar application of yeasts resulted in a significant increment of vegetative growth (including plant height, number of leaves, number of branches and fresh weight of plants) and yield of eggplant compared to control plants.

The results in Table (12) indicates that the shoot fresh weight were significantly improved by yeast inoculation of isolates (I1, I2, I3, I4, I5,) on tomato, pepper, and rice seedling varieties. The highest values recorded in rhizospheric soil yeast isolate (I5) are Pepper IM 42 (83.55%), Pepper Markofana (82.6%), Rice x-jigna (88.18%), Rice ediget (86.6%), Rice Getachew (92.38%), Tomato melksalsa (54.34%), Tomato kochero (61.76%) and Tomato miya (62.4%). This result is in harmony with Asma *et al*, (2013). The positive effects caused by the addition of yeast suspension in improving shoots characteristics might be due to the direct or indirect effect of the yeast throughout its ability in changing the environment of roots, or because the development of the yeast after its analysis in to wide groups of amino acids and vitamins.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The results can be concluded that yeasts effectively improved the growth of Tomato, Pepper, and Rice seedling varieties without causing any impact on the environment and on the ecology of other organisms. This study has clearly shown that different kind of yeasts that can be isolated from different sources and beverages are quite effective in improving and stimulating the growth (plant height, number of leaves, root length, number of node and internode, number of branches and fresh weight) of plants of varieties of Tomato. Pepper and Rice plant seedlings. Yeasts isolated from the Rhizospheric soil were more effective in plant growth promoting ability than other yeast isolates. It significantly enhanced the overall growth of the treated plants. The present study allowed the isolation and characterization of different yeast isolates and their ability and effectiveness to stimulate Rice, Tomato, and Pepper seedling growth. Plant growth promoting yeasts can be a true success story in sustainable agriculture.

6.2. Recommendations

Yeasts in the rhizosphere of different plants are diverse. So detail identification and characterization of those yeasts should be investigated.

The mechanisms and process that are involved in the regulation of plant growth by yeast isolates should be investigated and researched in the future.

On the basis of the promising findings presented in this study, future works will be conducted at field level to get a reliable result of yield.

Further researches need to be conducted toward the roles of yeasts in biological control. Formulation, mass culturing and molecular characterization is also the future activity of this research.

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Annexes

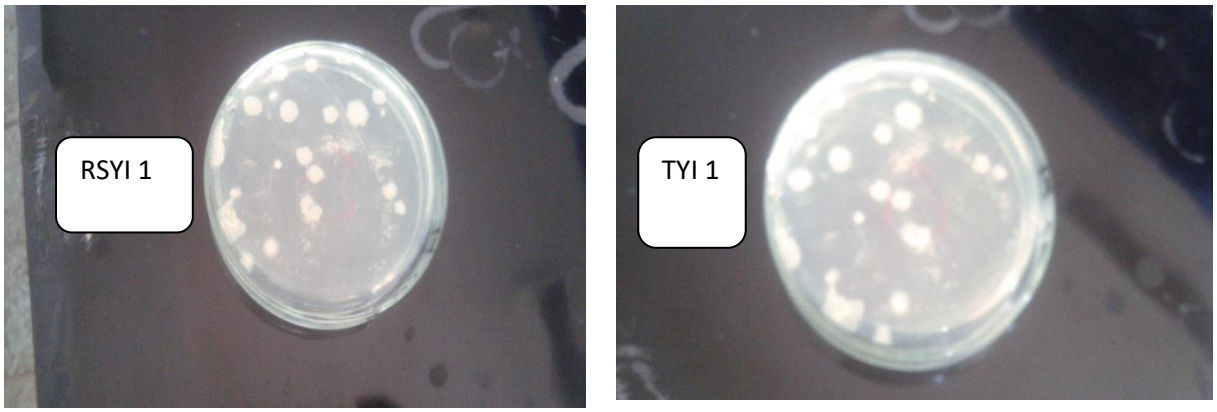

Figure 5 Morphological observation of some isolates, (RSYI 1=Rhizospheric soil yeast isolate 1, TYI 1=, Tella yeast isolate 1).

Declaration

I, the undersigned here by, declare that this thesis is my original work, has not been presented for a degree in any other university and that sources of materials used for this thesis have been duly acknowledged in compliance with internationally accepted practices, I have duly acknowledged and referenced all materials used in this work. I understand that non-adherence to the principles of academic honesty and integrity, misrepresentation/fabrication of any idea/data/fact/source will constitute sufficient ground for disciplinary action by the University and can also evoke penal action from the sources which have not been properly cited or acknowledged

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Name of Advisor

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Signature

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Name of Student

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Signature

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Date